In vitro ACTIVITY OF TOBRAMYCIN, AMILORIDES AND OTHER NON-ANTIBIOTICS AGAINST *Pseudomonas aeruginosa* AND *Burkholderia cenocepacia*

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CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree, nor has it been submitted as part of the requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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ABBREVIATIONS

arr	aminoglycoside response regulator
BCESM	*Burkholderia cepacia* epidemic strain marker
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane regulator
cfu	colony forming unit
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
IL	interleukin
MHB	Mueller-Hinton broth
MIC	minimum inhibitory concentration
mRNA	messenger RNA
MRSA	methicillin-resistant *Staphylococcus aureus*
NMDG	N-methyl-D-glucamine
PBPs	penicillin-binding proteins
QS	quorum-sensing
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
SD	standard deviation
SEM	standard error of the mean

ABSTRACT

Chronic respiratory infection, mainly caused by *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex, is the major cause of complications and eventually of death in patients with cystic fibrosis. These problems are exacerbated by drug resistance mechanisms induced in the infectious microorganisms, and by persistence of the microorganisms by sequestration in viscous mucus or biofilms. The sequestration prevents effective antibiotic access to the bacteria. Such problems have led to the search for alternative treatments and therapies, but none of these alternative techniques have yet been tested rigorously or successfully in clinical patients. In this project, we used a standard strain of *P. aeruginosa* (NCTC 10662) and a *B. cenocepacia* isolate from cystic fibrosis sputum to appraise tobramycin/amikacin efficacy in combination with clinically relevant concentrations of the adjunctive agents amiloride, benzamil hydrochloride, phenamil, salbutamol, verapamil, and amlodipine. Altered conditions in the cystic fibrosis lung were simulated by using different concentrations of sodium chloride, potassium chloride, sodium gluconate, D-mannitol, and N-Methyl-D-glucamine. Benzamil hydrochloride was the most potent additive compound against the organisms tested; enhancing the antibacterial effect of tobramycin. A sub-inhibitory concentration of amlodipine was only marginally useful, even though its minimum inhibitory concentration (MIC) against both microbes was the lowest of all the non-antibiotic compounds tested. Conversely, salbutamol, verapamil, and amlodipine were antagonistic in some combinations with tobramycin. Amikacin was generally more potent than tobramycin. Sodium and potassium chlorides and sodium gluconate increased the tobramycin MIC up to 8-fold at salt concentrations from 50–400 mM. This antagonistic effect of cations appeared to be partially reversed by adding amiloride, verapamil, or salbutamol. This study needs to be extended by further assays with more clinical isolates, but it has shown that non-antibiotic adjunctive agents can be used with antibiotics to produce effective results *in vitro*; and potentially *in vivo* as an alternative regime for the treatment of chronic airway infections in cystic fibrosis patients.